contrast, PBZ maintained its protective effect if given before blockade although 'acute' traumatic deaths incident to drumming increased. PBZ pretreatment had no effect on pre-shock or post-shock carbon clearance activity either in the intact rat or in the *in situ* perfused liver. The results indicate that in the rat carbon clearance activity is not well correlated with the protective action of PBZ in traumatic shock.

We express our thanks to Smith, Kline and French Laboratories, Philadelphia, Pa., for phenoxybenzamine hydrochloride (Lot 127-DI-(1)) used in this study.

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Effects of 1, 3, 5, (10), 16 Estratetraen-3-ol on Mammalian L-Fibroblasts *in vitro*.* (29367)

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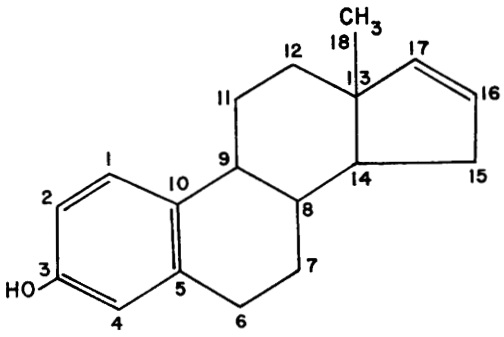
Previous investigations in this laboratory have revealed that concentrations of one-two ppm of 1, 3, 5 (10), 16-estratetraen-3-ol (coded and referred to hereinafter as chemical #742) when used as a culture medium for fish and/or frog embryos caused (tumors) hyperplasia in the tails of these embryos. Preliminary observations of the effects of #742 on cultures of pig kidney cells indicated that mitosis was accelerated.

Chemical #742 is a synthetic Δ^{16} steroid chemically related to the androgens. It was prepared by Huffman(1) from estradiol-3, 16. The chemical formula is



Materials and methods. Standardized stock cultures of L-fibroblasts (mouse) obtained from Dr. Franklin Leach, Oklahoma State University, were cultured in Eagle's medium, supplemented with calf serum, at 37.5°C. Cell suspensions for counting and/or transfer were formed by the use of a "policeman" and vigorous pipette action (20 ×).

Chemical #742 was supplied dissolved in

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propylene glycol, 1 mg/ml, by Dr. Max N. Huffman of the Lasdon Foundation. Dilutions were made with triple, glass-distilled water. Aliquots of the dilute solution were then added to the test cultures at time of transfer to produce the concentration desired. Similar aliquots of water alone were added to control cultures. Propylene glycol alone was also used as a test agent.

To study the cells microscopically they were grown on cover glasses placed in the bottom of petri dishes to which 10 ml of cell suspension and test chemical were added. After 72 hours' incubation adherent cells on the cover-glasses were fixed and stained in acetoorecein 30 minutes, rinsed in xylol and mounted in picolyte. Each dilution of test material was replicated at least 3 times and several slides(4-5) were prepared from each culture. One hundred and fifty-two slides were prepared and studied.

The mitotic index, or percentage of cells in division, was determined by dividing total number of cells in division by total number of cells observed (at least 1000)(2). Coverglasses were divided into 4 quarters and random fields were selected in each quarter for counting.

At least 3 morphologically different cell shapes as well as multinucleate cells were observed in both test and control cultures. The percentages of the multinucleate and of the various cell forms were determined in the same manner as the mitotic index using the same slides.

To substantiate the mitotic index findings from the stained slides, comparative counts of the cell numbers produced in test cultures were made. A 30 ml suspension of cells and media was prepared and divided into 3 equal aliquots. Propylene glycol was added to one aliquot. Chemical #742, in propylene glycol, was added to another, and sterile distilled water was added to the third. Each of these aliquots was then divided equally into 3 one-ounce culture bottles and incubated for 72 hours. All cells were then "policed" or scraped off the glass and suspended in the medium. Hemocytometer counts (white cell) were then made on each culture while in suspension. Averages were determined for the 3 cultures of each test substance.

TABLE I. Mitotic Index = % of Cells in Mitosis.

Control	3.64
.5 ppm #742	4.50*
1.0 " "	6.60*
5.0 " "	6.06*
10.0 " "	11.14†
20.0 " "	13.64†‡
2% propylene glycol (= that in 20 ppm #742)	7.2 ‡
40.0 ppm #742	lethal
4% propylene glycol (= that in 40 ppm #742)	"

* Not a significant increase when compared with control. (χ^2)
† A significant increase when compared with control. (χ^2)
‡ Difference between these two significant. (χ^2)

TABLE II. Cell Counts.

1.	Control	83.5/ml
2.	20 ppm #742	380.0/ml
3.	2% propylene glycol	215.0/ml

Each count above represents an avg of 3 counts each made from identically treated cultures in triplicate.
No. 2 and No. 3 each compared to No. 1 are statistically significant. (χ^2)
No. 2 and No. 3 compared to each other are statistically significant. (χ^2)

Results. Table I indicates the comparative mitotic indices as determined from the 152 slides. No one stage of mitosis appeared to be more frequent in either treated or controls. #742 appears to cause an increase in the mitotic index.

Table II shows the relative numbers of cells produced as shown by the hemocytometer counts. Both propylene glycol and #742 seemed to cause an increase in cell number.

Table III presents the relative abundance of multinucleate cells as observed in the various cultures. The vast majority of cells

TABLE III. Relative Percentage of Multinucleate Cells.

	%
Control	3.88
.5 ppm #742	5.80*
1.0 " "	6.30*
5.0 " "	4.10*
10.0 " "	2.10†
20.0 " "	2.70†
2% propylene glycol	2.57†

* Not significant difference from control. (χ^2)
† Significant difference from control. (χ^2)

TABLE IV. Relative Abundance of Morphological Cell Types.

	Type I	Type II	Type III
	——— % ———		
Control	52.57	37.62	9.79
.5 ppm #742	26.55	62.71	10.73
1.0 " "	20.37	63.70	15.92
5.0 " "	13.85	49.86	51.80
10.0 " "	8.30	16.60	75.09
20.0 " "	2.97	18.85	78.39
2% propylene glycol	25.23	62.39	12.39

termed "multinucleate" were bi-nucleate. However, cells with as many as 7 clearly discernible nuclei were observed. Cells in telophase were separated from binucleate forms on the basis of the cytoplasmic constriction and on the location and appearance of the nuclei themselves. Although the total number of cells increased in 10 and 20 ppm of #742 and in propylene glycol, the relative abundance of multinucleate cells decreased.

Table IV presents the relative abundance of morphological cell types as found in the various cultures. In general, 3 cell forms were observed: one, (Fig. 1), a large rounded cell with a relatively large amount of cytoplasm and few cytoplasmic processes; two, (Fig. 2), a smaller, stellate cell with definite cytoplasmic projections usually at opposite poles; three, (Fig. 3), a small fusiform cell. The relative abundance of these cell forms can be seen in the photographs and in Table IV. Chemical #742 appears to have a selective effect on which cell type survives best. Type III seems to be favored.

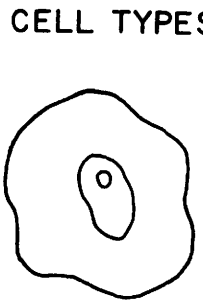

Figure 1. Type I

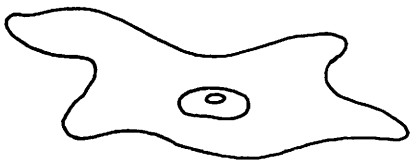

Figure 2. Type II

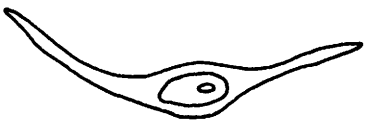

Figure 3. Type III

Discussion. It is not a safe assumption to consider fibroblasts in tissue culture as fully differentiated morphologically similar cells (3). Cells in culture tend to lose differentiation, not gain it.

Transformation of cell forms from one type to another have been frequently described(4, 5,6). It is probably not safe to assume that the populations of any given cell line propagated in tissue culture are or will remain uniform in cell morphology. This is demonstrated in our study of cell morphology distribution (Table IV and photographs). Chemical #742 although stimulating an increase in cell number also appears to have a selective influence on cell morphology.

Both chemical #742 and propylene glycol appear to stimulate cell division as shown by the data in Tables I and II. How much #742 alone affects mitotic activity cannot be determined from these data because propylene glycol is always present as the carrier for this compound. Neukomm(7) reported that propylene glycol affected growth when used as a solvent for methylcholanthyrene. In our laboratory, propylene glycol in concentrations of 0.1-0.2% seems to affect the production of melanin in fish embryos and at higher concentrations, 1.0-2.0 percent, seems to retard growth of the embryos and eventually to kill them. However, at no time has there been observed any evidence of tumorous or hyperplastic growth; nor have there been observed other phenomena of development which can be ascribed to an increase in mitotic activity brought about by the propylene glycol alone(8). So far, we have not determined whether there are any synergistic

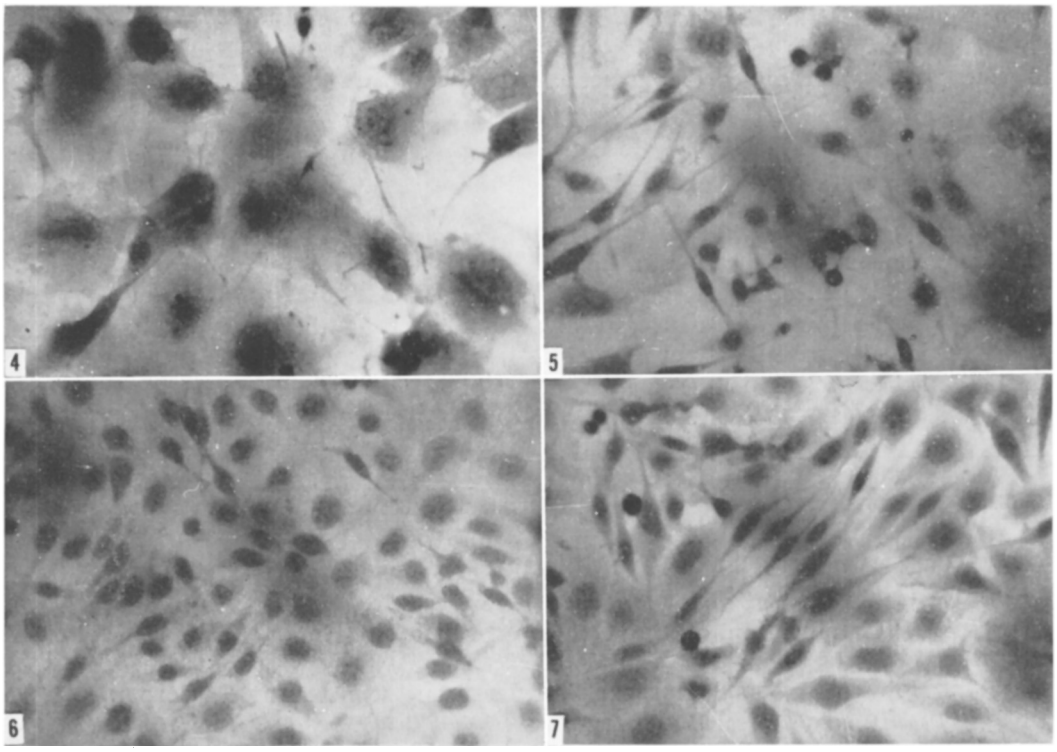

FIG. 4. Controls. FIG. 5. 10 ppm 742. FIG. 6. Propylene glycol equivalent to amount in 20 ppm 742. FIG. 7. 20 ppm 742.

effects due to interaction of propylene glycol and chemical #742.

Data in Table III indicate that there was an increase in the number of multinucleate cells present in the lower concentrations of #742 but at higher concentrations the number was decreased. Propylene glycol probably was responsible. Unfortunately, control studies using lower concentrations of propylene glycol were not made. The multinucleate cells were restricted to Type I for the most part with only a few cells of Type II showing more than one nucleus. Multinucleate cells of Type III were very rarely observed. The nuclei of the multinucleate cells were not necessarily all of the same size or shape. Often a large ovoid nucleus surrounded by several smaller nuclei was observed. Multinucleate cells may originate as a result of mitosis(9) or of abortive or delayed cytoplasmic division following mitosis. Some evidences of amitosis were observed in the cells studied herein.

There is perhaps a relationship between the abundance of multinucleate cells and those of different morphology or type. Rothchild(10) reported a hemolyzing effect of propylene glycol on erythrocytes. This is an effect on cell membranes which would influence cell morphology and perhaps account for the lack of multinucleate cells in the higher concentrations of propylene glycol and #742.

Table IV data show that there was a distinct change in cell morphology due to the presence of #742 in the culture medium. As the concentration of #742 increased the proportionate number of Type I and Type II cells decreased and Type III increased. Propylene glycol seemed to result in a decrease in Type I cells and an increase in Type II. Studies of the prepared slides suggest that perhaps Type III cells may originate from Type I and Type II cells.

None of the data herein presented or the observations made of the L-fibroblast cultures provide explanations of the hyperplastic growths formed in fish and frog embryos

when exposed to #742. The increase in cell division and changes in cell morphology may be clues as to what causes the malformations.

Summary. L-fibroblast cell cultures were treated with varying concentrations of 1, 3, 5, (10), 16-estratetraen-3-ol (#742) dissolved in propylene glycol. Propylene glycol (2%) was found to stimulate the mitotic rate. With #742 added the mitotic rate and total cell number were increased markedly. The presence of propylene glycol and #742 caused a distinct change in the morphology of the cells present in the cultures.

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Calcification of the Ovarian Follicle in Rats.* (29368)

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Parathyroid extract (PTE) under certain conditions can cause deposition of calcium in soft tissues. Calcification may occur in the cortex of the kidney(1,2,3,4,5), in the gastric mucosa and in a variety of other soft tissue sites(6) after adequate dosages of PTE. There are, however, no reports of PTE-induced calcification in the reproductive organs. This communication is concerned with PTE-induced calcification of the ovarian follicular apparatus of the rat.

Materials and methods. Immature female rats of the Wistar strain weighing 30-50 g were divided into 3 groups. The experimental group was injected subcutaneously with 40 units of "Parathyroid Extract" (Lilly) twice daily for 3 days. One group of controls was given PTE, inactivated by either pepsin-digestion or formalin according to the methods of Shetlar(3). This material was given subcutaneously in amounts and for periods of time comparable to the use of the active extract in the experimental group. A third group of female rats of comparable weight and age served as uninjected controls. All animals were maintained on a diet of commercially available rat pellets and tap water administered *ad libitum.*

The experimental animals were sacrificed 24 hours after the last injection by ether asphyxiation and were autopsied immediately. Samples of ovary, uterus, cervix, vagina, and kidney were removed from these animals as well as from the controls, fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned. Alternate sections were stained with either hematoxylin and eosin, the von Kossa technique, Alizarin Red S or the PAS technique. Metachromasia was studied in the tissues by staining with Thionin.

Results. A summary of the microscopic findings is presented in Table I. Calcium

* This work was derived in part from a dissertation for the degree of Doctor of Philosophy in the Department of Biology at New York University by H. S. Grob. Supported in part by a grant from Nat. Inst. Health, Cancer Research Training.

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